Acute Pulmonary Toxicity of Particulate Matter Filter Extracts in Rats: Coherence with Epidemiologic Studies in Utah Valley Residents

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Epidemiologic reports by C.A. Pope III et. al. demonstrated that in the Utah Valley, closure of an open-hearth steel mill over the winter of 1987 was associated with reductions in respiratory disease and related hospital admissions in valley residents. To better examine the relationship between plant-associated changes in ambient particulate matter (PM) and respiratory health effects, we obtained total suspended particulate filters originally collected near the steel mill during the winter of 1986 (before closure), 1987 (during closure), and 1988 (after plant reopening). PM subcomponents were water-extracted from these filters and Sprague-Dawley rats were intratracheally instilled with equivalent masses of extract. Data indicated that 24 hr later, rats exposed to 1986 or 1988 extracts developed significant pulmonary injury and neutrophilic inflammation. Additionally, 50% of rats exposed to 1986 or 1988 extracts had increased airway responsiveness to acetylcholine, compared to 17 and 25% of rats exposed to saline or the 1987 extract, respectively. By 96 hr, these effects were largely resolved except for increases in lung lavage fluid neutrophils and lymphocytes in 1986 extract–exposed rats. Analogous effects were observed with lung histologic assessment. Extract analysis using inductively coupled plasma–mass spectroscopy demonstrated in all three extracts nearly 70% of the mass appeared to be sodium-based salts derived from the glass filter matrix. Interestingly, relative to the 1987 extract, the 1986/1988 extracts contained more sulfate, cationic salts (i.e., calcium, potassium, magnesium), and certain metals (i.e., copper, zinc, iron, lead, strontium, arsenic, manganese, nickel). Although total metal content was ≤1% of the extracts by mass, the greater quantity detected in the 1986 and 1988 extracts suggests metals may be important determinants of the pulmonary toxicity observed. In conclusion, the pulmonary effects induced by exposure of rats to water-based extracts of local ambient PM filters were in good accord with the cross-sectional epidemiologic reports of adverse respiratory health effects in Utah Valley residents. *Key words:* filter extraction, lung inflammation, lung injury, metals, particulate matter (PM), pulmonary toxicity, rats, steel mill, total suspended particulates (TSP), Utah Valley. — *Environ Health Perspect* 109(suppl 3):395–403 (2001).
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The National Research Council on Research Priorities for Airborne Particulate Matter has emphasized the need to establish biologic plausibility with regard to the health effects of particulate matter (PM) as described by many recent epidemiologic reports [for reviews see (*1*,*2*)]. One such report published by Pope in 1989, was a seminal study associating Utah Valley hospital respiratory admissions with PM_{10} levels (PM < 10 μm) from April 1985 to February 1988 (*3*). In the Utah Valley, PM concentrations often increase during winter months when temperature inversions trap emissions in stagnant air near the valley floor. The period investigated by Pope was inclusive of intervals of closure and operation of the Geneva Steel Mill. While operational, this open-hearth plant contributed up to 80% of all industrial-related PM in the Utah Valley. But on 1 August 1986 the steel mill closed because of a labor strike. It remained closed for 1 year until reopening under new ownership on 1 September 1987. These events provided a fortuitous opportunity to examine whether morbidity among those in the affected area could be temporally correlated with PM excursions. Using ambient PM_{10} measurements from the sampling site in Lindon, Utah, near the steel mill, Pope reported over 80% of monthly hospital admissions for respiratory causes, particularly among children with lung or airway impairments, were related significantly to the mean as well as to the peak ambient PM_{10} levels for both the immediate and previous months. Furthermore, these hospital admissions decreased soon after plant closure; they increased again when the plant reopened, as did public complaints of respiratory discomfort. However, other admissions and time periods did not exhibit any correlation nor did hospital admissions in areas not affected by the Geneva Steel Mill show correlations with PM_{10} concentrations.

Although the operating status of the Geneva Steel Mill provided a remarkable opportunity to investigate the link between wintertime Utah Valley PM excursions and respiratory disease in valley residents, efforts are still necessary to enhance the biologic plausibility of this potential cause-and-effect relationship. Ideally, one would seek to establish the inherent toxicity of ambient particles collected in the valley over the time periods under investigation. Unfortunately, no bulk air-sampling systems (e.g., high-volume cyclones) were in place at the time. Alternatively, however, the Utah Division of Air Quality had archived daily PM filters collected at the Lindon monitoring site during the winter months of the year before and during closure of the steel mill, as well as the year after the plant reopened. Through collaboration with Air Quality personnel, official ambient high-volume (hi-vol) PM filters were obtained from these time periods. PM subcomponents were water-extracted from the filters, then using equivalent masses of the extracted material, we established the relative pulmonary toxicity of the extracts in young adult rats. Our hypothesis was, on an equal mass basis, material extracted from filters collected during plant closure would be less toxic. We subsequently characterized the water-based extracts in terms of their elemental composition, pH in suspension, general solubility, and endotoxin content. With this database we attempted to establish whether the water-based extracts yielded *in vivo* animal toxicity data coherent with the aforementioned epidemiologic observations, and whether there were biologic or physicochemical differences in the extracts that may relate to their inherent toxicity.

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Materials and Methods

Preparation of Filter Extracts

Archived glass hi-vol filters collected at Lindon for monitoring Utah Valley ambient total suspended particulate (TSP) concentrations were obtained from the Air Monitoring Center, Utah Division of Air Quality (Salt Lake City, UT, USA). These filters, collected under federal guidelines (*4*), had been stored, folded PM-side inward, in plastic sleeves at room temperature and humidity after original use for state monitoring purposes. Twelve filters from each of three consecutive winters were selected. The first winter, defined as January–March of 1986, included filters collected prior to closure of the steel mill. Filters from January to March of 1987 were collected during closure of the mill, and filters from January to March of 1988 were collected after the steel mill had reopened. Because of inherent difficulties with removing particulate material directly from hi-vol filters, subcomponents of the PM were extracted via agitation of individual 8 × 10-in. filters in 50-mL conical polypropylene centrifuge tubes with 40 mL of deionized water for 96 hr. Filters were drained and removed, and the remaining liquid was centrifuged at 2,500×*g* for 30 min to pellet relatively insoluble material. For each year, supernatants for the respective years were pooled and then lyophilized. The resultant three samples are referred to in the remainder of the text as 1986, 1987, and 1988 extracts.

Animals

Sixty-day-old, male CD (Sprague-Dawley) rats [Crl:CD(SD)BR rats, Charles River Laboratories, Raleigh, NC, USA] were maintained in an AAALAC-approved animal facility; animals were housed in pairs within polycarbonate cages containing laboratory-grade pine shaving (North Eastern Products Corp., Warrensburg, NY, USA). All experimental protocols were approved by the Institutional Animal Care and Use Committee. Facility rooms were regulated at a constant temperature (22 ± 2°C) and humidity (50 ± 10% relative humidity) and 12-hr light:12-hr dark cycles. Food (rodent chow: PMI, Nutritional International, Brentwood, MO, USA) and water were available *ad libitum*. For each experiment, rats were randomized into groups such that body weights (mean ± SE) of the groups were equivalent at the onset of the exposure.

Intratracheal Instillation

During brief anesthesia with halothane vapor (Aldrich Chemical Co., Milwaukee, WI, USA), the rats were transorally intubated using a fiberoptic laryngoscope and a 16-gauge Angiocath catheter (Becton-Dickson, Sandy, UT USA) as an endotracheal tube. Saline or extract suspensions were instilled through the endotracheal tube using a syringe and a smaller injection catheter (Angiocath, 18 gauge, Becton-Dickinson). All suspensions were prepared in the same sterile saline (Fugisawa, Deerfield, IL, USA). After placing each extract into saline, we observed that the 1986 and 1988 preparations turned brownish in color, whereas the 1987 preparation was more tan in appearance. We also observed the extracted material did not dissolve completely at the concentrations used. Therefore, prior to instillation, all preparations were sonicated in a water bath for 10 min to maximize resolubilization of the lyophilized material. Visibly, comparable amounts of a small but finite amount of extract would still not redissolve without acidification. To preserve the nature of the original extract, the instillate was not acidified but rather instilled with small amounts of nondissolved materials. Frequent remixing was used to ensure appropriate dosing of the extracts.

Experimental Design

Two separate experimental phases formed the basis of these studies. The first phase was designed to determine the relative toxicity of each extracted sample, in that all rats were instilled with an equal mass of extract (see experiment 1 below). The second phase, performed after elemental analysis, was designed to evaluate dose–response characteristics of the 1986 plant on extract (experiment 2A) and the 1987 plant off extract (experiment 2B).

Experiment 1

Extracts were reconstituted in sterile 0.9% saline at a concentration of 8.33 mg/mL. Based in part on experiences with other PM samples, rats (n = six/group) were intratracheally instilled with either 0.3 mL sterile saline or 0.3 mL of one of the three extracts resuspended in saline (i.e., 8.33 mg extract/kg body weight or ≈ 2.5 mg/rat). At 24 and 96 hr postinstillation, these animals were evaluated for changes in airway responsiveness and bronchoalveolar lavage fluid indices of injury and inflammation. An independent replicate experiment was performed using an additional six rats/group with identical instillation conditions for a 24-hr postinstillation assessment. For lung histologic evaluation an additional three rats/group were intratracheally instilled (2.5 mg/rat) and used only to assess pathologic changes at 24 or 96 hr postinstillation.

Experiments 2A and 2B

After elemental analysis and metal determination, experiment 2A animals were instilled with either 0.3 mL saline intratracheally or 0.3 mL of the plant on 1986 extract prepared to administer 0.25 mg/rat (i.e., 10% of the original dose) and 1.0 mg/rat (i.e., 40% of the original dose). In experiment 2B, rats were instilled with either 0.3 mL saline or a suspension of the plant off 1987 extract prepared to administer 5.0 mg/rat (i.e., double the initial dose). Airway responsiveness testing and bronchoalveolar lavage (BAL) fluid cellular and biochemical indices were evaluated at 24 hr postinstillation as indicated below.

Bronchoprovocation Testing

Nonspecific airway responsiveness was assessed in rats using provocation testing as has been previously described (*5*). The rats were anesthetized with urethane (Sigma Chemical Co., St. Louis, MO, USA) at 1 g/kg (intraperitoneal) for surgical implantation of an indwelling jugular venous catheter followed by transoral intubation for ventilation after paralysis with succinylcholine (GlaxoSmithKline Co., Research Triangle Park, NC, USA) at 2.5 mg/kg (intramuscular). Placed in dorsal recumbency, rats were ventilated at 90 breaths/min with a tidal volume of 7.5 mL/kg (previous studies determined these settings maintained adequate arterial blood gas pH, pO_2, and pCO_2 values). Airway opening pressure (Pao), tidal volume, and flow signals (calculated by electronic differentiation of volume) were the primary recorded measures. Bronchoprovocation was achieved by sequential intravenous infusion of saline and acetylcholine (ACh) (Sigma Chemical Co.). After obtaining baseline measurements of Pao × 1 min, a saline infusion was administered at 0.04 mL/min × 1 min, followed by ACh infusion beginning at 0.04 mL/min, with the delivery rate doubling every 2 min, up to a 0.64 mL/min flow rate. The concentration of ACh was body-weight adjusted for all animals (900 µg/mL/kg), resulting in an average ACh dose of 2.25 mg/kg in a total volume of 2.5 mL saline. Software developed with the LABVIEW graphical programming language (National Instrument Corp., Austin, TX, USA) was used for data aquisition and storage.

Bronchoalveolar Lavage

Following completion of the airway responsiveness testing, while still anesthetized, rats were euthanized via exsanguination. Through the endotracheal tube the lungs were lavaged 3 times using the same volume of calcium/magnesium (Ca/Mg)-free phosphate-buffered saline (28 mL/kg body weight). Total cell counts of the BAL fluid were performed using an electronic cell counter (model ZBI; Coulter Electronics, Hialeah, FL, USA). Differential cell counts were performed on cytocentrifuge preparations (Cytospin Model II; Shandon Pittsburgh, PA, USA) stained with Diff-Quick (American Scientific Co., Sewickley, PA, USA) by enumeration of 200

cells per slide. The BAL fluid was centrifuged at 500×*g* for 10 min at 4°C; supernatants were analyzed for total protein (TPR) (Coomassie plus reagent; Pierce and Co., Rockford, IL, USA) and lactate dehydrogenase (LDH) (Assay kit 228; Sigma Chemical Co.) concentrations using an automated centrifugal spectrophotometer (Cobas Fara II; Hoffman-LaRoche, Branchburg, NJ, USA).

Pathology

Left lungs from a subset of saline- or extract-exposed rats (three rats/group) were infused intratracheally with filtered 4% paraformaldehyde (prepared in 1× phosphate-buffered saline, pH 7.2). The inflation volume, 11.2 mL/kg body weight, was calculated by assuming total lung capacity (TLC) was approximately 28 mL/kg and 40% of TLC was related to the capacity of the left lung lobe. Inflated lungs were stored at 4°C until paraffin embedment. Midsagittal sections 4 µm thick were stained with hematoxylin–eosin (HE) for evaluation by light microscopy. Lung pathology was evaluated in a blinded manner regarding incidence and severity of the lesions; changes were classified as degenerative, proliferative, or inflammatory.

Statistical Analyses

Data were analyzed with analysis of variance (ANOVA) using Scheffe's posttest correction for multiple comparisons or with a *t* test for single comparisons as appropriate. For all analyses, group differences were considered significant if the test statistical type I error was less than 0.05 (i.e., $p < 0.05$) (*6*).

Extract Analysis

Endotoxin analysis. An aliquot of each extract was prepared in preservative-free saline at a concentration of 8.33 mg/mL. The endotoxin content of each sample was determined in duplicate using the limulus amebocyte lysate assay (gel clot method) employing *Escherichia coli* endotoxin as the control standard (Associates of Cape Cod, Inc., Falmouth, MA, USA).

Extract analytical methods. Filter extracts were resuspended in 1 M HCl and analyzed by inductively coupled plasma atomic–emission spectroscopy (ICP–AES) (Model P40, Perkin Elmer, Norwalk, CT, USA) for preliminary evaluation of their elemental content in terms of six metals, using an analytical protocol closely following U.S. Environmental Protection Agency (U.S. EPA) method 200.7. Extract samples were also evaluated for content of 44 elements using neutron activation analysis (NAA). NAA parameters included 20-sec and 6-hr irradiation periods with decay signals monitored using an Ortec 38% GeLi detector (Ortec, Oak Ridge, TN, USA) coupled to a computerized gamma detection system (Nuclear Engineering Department, N.C. State University, Raleigh, NC, USA). Finally, water-resuspended and 1 M HCl-dissolved samples were evaluated for 28 elements using inductively coupled plasma–mass spectroscopy (ICP–MS) (Elan 6000, Perkin-Elmer), by an analytical protocol closely following U.S. EPA method 6020. Briefly, the ICP–MS measures stable (nonradioactive) isotope content. Commercially available, multielement standards from independent sources were used for calibration (CL-CAL-2; SPEX Industries, Inc., Edison, NJ, USA) and quality control (QC) checks (LCAL6020-100; VHG Labs, Inc., Manchester, NH, USA). In addition to the method 6020 elements, silicon and sulfate levels were measured; calibration and QC standards were purchased from the same vendors as specified above for the multielement standards. For ICP–MS, all samples were determined in duplicate.

Results

Experiment 1

By 24 hr after intratracheal instillation of the 1986 or 1988 extracts (plant on years), rats developed acute pulmonary injury characterized by significant increases in BAL fluid concentrations of LDH (159 and 153%, respectively) and TPR (60 and 76%, respectively) (Table 1). Exposure to the 1986 or 1988 extracts also resulted in significant increases in the total number of identifiable cells recovered in the lavage fluid (Table 1). These effects were largely driven by a robust neutrophilic inflammatory response (i.e., up to 22-fold increase in neutrophil numbers) (Figure 1A). Relative to the saline-instilled controls, rats exposed to the 1987 (plant off) extract failed to develop significant biochemical or cellular lavage fluid changes. By 96 hr, lavage fluid concentrations of LDH and TPR were reduced, consistent

Table 1. Comparison of BAL fluid biochemical and cellular indices (mean ± SE) and airway responsiveness to ACh in saline- or extract-exposed rats (2.5 mg/rat).

		Extract		
Parameters (units)	Saline	1986	1987	1988
24 hr postinstillation				
BAL fluid indices	(*n* = 12)	(*n* = 12)	(*n* = 12)	(*n* = 12)
LDH (U/mL)	26.2 ± 4.1	67.8 ± 9.7*	24.9 ± 3.9	66.3 ± 7.8*
Total protein (µg/mL)	197 ± 22	315 ± 44*	206 ± 10	346 ± 13*
Total cells × 10^3/mL	62 ± 10	440 ± 28*	97 ± 8	316 ± 20*
ACh EC_{150} arithmetic mean	6.0 ± 1.0	5.4 ± 1.0	6.9 ± 1.0	4.6 ± 0.8
Upper 95% CI	8.2			
Lower 95% CI	3.7			
% < lower 95% CI	17% (2/12)	50% (6/12)	25% (3/12)	50% (6/12)
96 hr postinstillation	(*n* = 6)	(*n* = 6)	(*n* = 6)	(*n* = 6)
BAL fluid indices				
LDH (U/mL)	14.5 ± 6.5	31.1 ± 8.3	18.2 ± 3.5	22.3 ± 6.5
Total protein (mg/mL)	138 ± 12	197 ± 24	131 ± 5.3	162 ± 8.2
Total cells × 10^3/mL	62 ± 6.1	106 ± 12*	45 ± 1.6	66 ± 5.0
ACh EC_{150} arithmetic mean	6.2 ± 1.3	4.5 ± 1.5	5.0 ± 0.8	5.1 ± 1.0
Upper 95% CI	9.4			
Lower 95% CI	2.9			
% < lower 95% CI	17% (1/6)	33% (2/6)	0% (0/6)	17% (1/6)

*Significantly different value than saline-instilled rats at the corresponding time point.

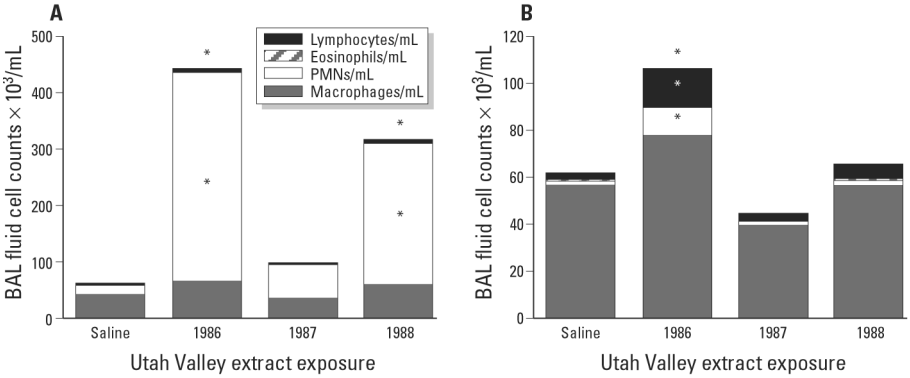

Figure 1. (*A*) Comparison of the changes in BAL fluid cell numbers 24 hr after intratracheal instillation of either saline or water-derived TSP filter extracts (2.5 mg extract/rat) from filters collected in the Utah Valley during the winter of 1986 (plant on), 1987 (plant off), or 1988 (plant on again). Asterisk (*) indicates significantly different from saline-exposed rats (*n* =12 rats/group). PMN, polymorphonuclear leukocyte. (*B*) Comparison of the changes in BAL fluid cell numbers 96 hr after intratracheal instillation of either saline or water-derived TSP filter extracts (2.5 mg extract/rat) from filters collected in the Utah Valley. Asterisk (*) indicates significantly different from saline-exposed rats (*n* = 6 rats/group).

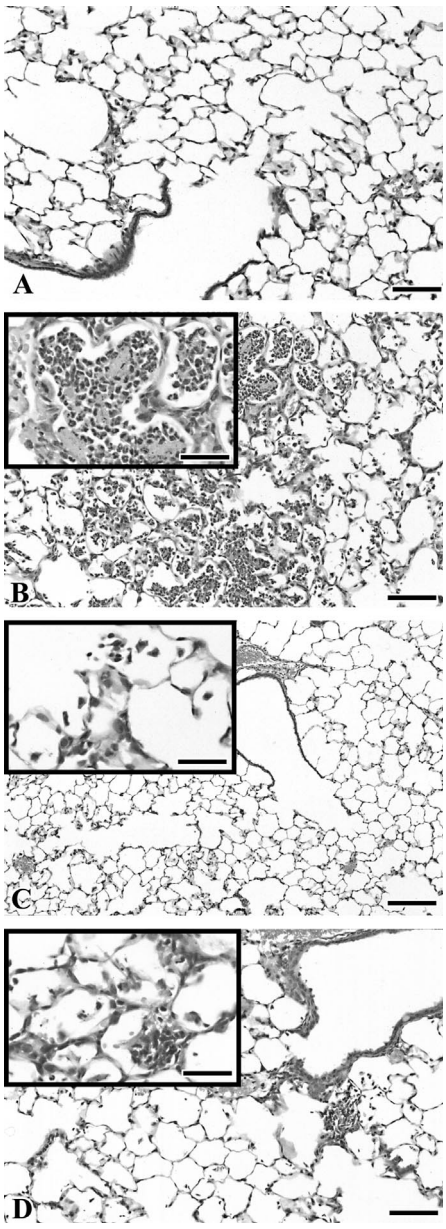

Figure 2. (*A*) Lung from saline-instilled control rat, 24 hr postinstillation. Relatively normal-appearing lung with mild centriacinar zone changes present consisting of minimal increases in alveolar macrophage numbers and an occasional neutrophil. Hematoxylin–eosin. Bar = 75 µm. (*B*) Lung from 1986 extract–exposed rat (2.5 mg) 24 hr postinstillation. Moderately severe locally extensive alveolitis is present. Insert depicts pleocellular inflammatory exudate consisting of PMNs and extract-laden macrophages. Hemorrhage is present in the intraalveolar spaces. There is mild thickening of alveolar septae in association with the inflammatory exudate. Hematoxylin–eosin. Bar = 150 µm. Insert bar = 75 µm. (*C*) Lung from 1987 extract–exposed rat (2.5 mg) 24 hr postinstillation. Minimal centriacinar hypercellularity is observed. Insert shows neutrophils and macrophages along an alveolar duct. Hematoxylin-eosin. Bar = 150 µm. Insert bar = 75 µm. (*D*) Lung from 1988 extract–exposed rat (2.5 mg) 24 hr postinstillation. Mild centriacinar inflammatory infiltrate is evident. Insert depicts infiltrate consisting of extract-laden macrophages and neutrophils. Hematoxylin–eosin. Bar = 75 µm. Insert bar = 35 µm.

with ongoing resolution of overt lung injury (Table 1). Yet at 96 hr, rats exposed to the 1986 extract still had a significantly greater number of cells in the lavage fluid owing to persistent increases in neutrophils (7.5-fold increase), as well as significant increases in lymphocytes (6.2-fold increase) (Figure 1B).

Analogous effects were observed with lung histologic examination. At 24 hr, rats exposed to the 1986 or 1988 extracts exhibited significant lung pathology (Figure 2). Briefly, rats exposed to the 1986 extract developed moderately severe alveolitis that included evidence of hemorrhage into the alveolar spaces, and rats exposed to the 1988 extract had evidence of mild centriacinar inflammation. By 96 hr after exposure to the 1986 or 1988 extracts, although changes were qualitatively similar, considerable repair had occurred (data not shown).

Differences in nonspecific airway responsiveness were evaluated by calculating the effective ACh concentration required to induce a 50% increase in baseline Pao measurements (EC_{150}) for individual rats. Subsequent comparisons of the group mean EC_{150} values failed to reveal significant differences in any of the extract-exposed rats relative to the saline-instilled control rats at either 24 or 96 hr postinstillation. In an effort to discriminate individual hyperresponsive rats within each exposure group, we elected to define a normal range of airway responsiveness by establishing a 95% confidence interval (CI) about the EC_{150} for the saline-exposed rats for the corresponding time period after instillation (Table 1). When the 95% CI was established for the 24-hr data set, we observed 50% of rats exposed to the 1986 or 1988 extracts had EC_{150} values that fell below the lower 95% CI (i.e., were deemed hyperresponsive). By comparison, only 17% of the saline-exposed rats and 25% of the 1987 extract–exposed rats had EC_{150} values that fell below the lower 95% CI. By 96 hr postinstillation only 25, 0, and 17% of the 1986, 1987, and 1988 extract–exposed rats, respectively, compared to 17% of the saline-exposed rats were hyperresponsive by this definition. These data suggest individual rats exposed to 1986 or 1988 extracts developed transient increases (i.e., at 24 hr postinstillation) in their nonspecific airway responsiveness.

Extract Analysis

Having confirmed the pulmonary toxicity resulting from exposure to equal mass amounts of the 1987 extract was consistently less than that induced by exposure to the 1986 and 1988 extracts, extracts were characterized in terms of their pH in suspension, endotoxin content, and elemental composition and solubility. For all extracts, when placed in saline, the aqueous suspensions became relatively alkaline (i.e., pH 9–10). The endotoxin concentration reported for the extracts was 1.5 endotoxin units (EU)/mg of 1986 extract, 0.12 EU/mg of 1987 extract, and 6.0 EU/mg of 1988 extract. Although there appeared to be less endotoxin in the 1987 extract, even the amount of endotoxin instilled in rats receiving the 1988 extract (i.e., 6 EU/mg × 2.5 mg = 15 EU/rat) is considerably less than that typically associated with induction of lung inflammation in healthy CD rats (*7*).

One PM subcomponent we were particularly interested in was transition metal content. Steel mills and their associated combustion processes are likely to contribute considerable PM into the associated airshed—PM that would be expected to have relatively high amounts of metal. Initially, extracts were dissolved in 1 M HCl and analyzed for copper (Cu), iron (Fe), lead (Pb), nickel (Ni), vanadium (V), and zinc (Zn) using ICP–AES methodologies. Results demonstrated several of the metals were present in greater concentrations in the 1986 and 1988 extracts; however, metal concentrations (expressed as micrograms metal per gram of extracted mass) were exceedingly low compared to other ambient TSP samples we have similarly analyzed (*8*). In fact, metal content represented less than 1% of the extracted mass. We therefore attempted to further define the elemental composition of the extracts using NAA methodologies. Unfortunately, of the 44 elements analyzed, 10–13 were below detectable limits and 12–14 elements (including several of the metals of interest) were not interpretable because of high sodium levels in the extracts. In addition, neither sulfur (and thus sulfate) nor lead determinations were possible with NAA.

As a consequence, we developed a procedure to analyze the extracts using ICP–MS capable of nearly 1,000 times greater sensitivity for most elements than is possible with ICP–AES evaluation. Extracts were evaluated for 28 different elements. In addition, because we observed the extracts failed to resolubilize completely when placed in 0.9% saline, we also determined which elements in the lyophilized samples were readily soluble in water. To this end, extracts were suspended in water or in 1 M HCl, samples centrifuged, and supernatants analyzed using ICP–MS. Results again indicated the pH of the water-resuspended samples was 9–10. Of the 28 elements determined using ICP–MS under method 6020, 14 elements were below detection limit, quantitation was problematic due to Na- and Cl-based interferences, or results failed method 6020 quality control checks at the levels measured (again, mostly because of polyatomic interferences). The two most abundant elements present were sodium and

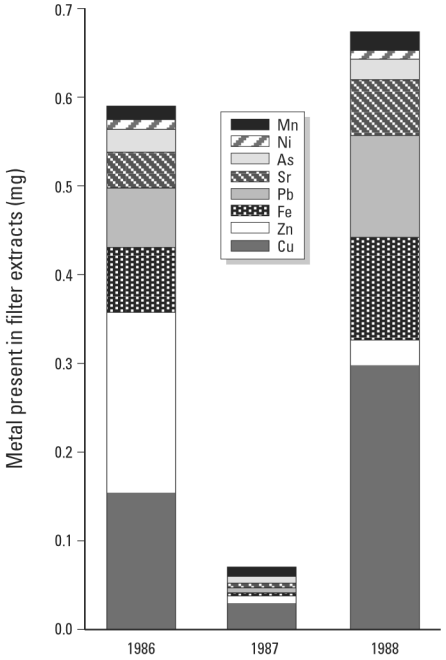

Figure 3. Comparison of the total amount of key metals present in these extracts that were obtained by water extraction of 12 individual TSP-containing glass hi-vol filters per year. The filters were collected in the Utah Valley during the winter of 1986 (plant on), 1987 (plant off), and 1988 (plant on again).

sulfur. Ambient particulate sulfur content is almost all (> 90%) in the form of sulfate (*9*). Of interest, results indicated that for 13 elements, concentrations were greater in the 1986 and 1988 extracts. In descending order of detection, these included (SO_4^{2}), Ca, potassium (K), Mg, Cu, Zn, Fe, Pb, strontium (Sr), arsenic (As), manganese (Mn), Ni, and possibly selenium and barium, although the levels of these latter two elements were exceedingly low. The total quantity of individual metals detected in the extracts has been depicted in Figure 3 for each year. We also calculated the relative water solubility of these elements (i.e., the amount of element present in the water-resuspended sample divided by the amount in the corresponding acid-resuspended sample × 100%). The water solubilities were as follows: SO_4^{2-} (100%), Ca (42–94%), K (86–98%), Mg (3–67%), Cu (61–73%), Zn (0.2–9%), Fe (0–33%), Pb (4–60%), Sr (25–94%), As (82–100%), Mn (9–21%), and Ni (32–93%). The wide range in solubilities observed was largely due to the 1987 extract in that, although this extract had the lowest quantity of most elements, many of the elements present were somewhat more soluble.

Experiments 2A and 2B

To follow up on the initial study comparing the relative pulmonary toxicity associated with exposure to 2.5 mg/rat of the 1986–1988 filter extracts, two separate, but analogous bioassays were conducted to expand our understanding of the nature of year-specific responses. Experiment 2A was designed to ascertain whether there existed a dose-dependent response with the more toxic 1986 (plant on) extract. The doses chosen for follow-up of the 1986 extract were 10% (0.25 mg/rat) and 40% (1.0 mg/rat) of the original dose. As the maximum effects in the initial study were found to be at 24 hr post-instillation, only that time point was evaluated in these follow-up studies. Data indicated that even at these reduced exposures, lavage fluid cell counts were significantly increased, again reflecting increases in neutrophils (24- and 57-fold, respectively, with the 10 and 40% doses). Results are indicative of significant dose dependency of the inflammatory response to the 1986 extract (Table 2). Increases in lavage fluid LDH (188%) and protein (54%) concentrations showed analogous dose trends, although the increases in protein were not statistically significant. Other lavage fluid cell types, including macrophages, were unchanged, and there were no extract-induced changes in airway responsiveness to ACh.

In experiment 2B, we attempted to evaluate whether exposure to increased amounts of the 1987 (plant off) extract would result in overt toxicity. Because of the finite amount of remaining extract, we chose to use a single exposure double that of the initial dose (i.e., rats were instilled with 5.0 mg/rat). As shown in Table 3, doubling the 1987 extract exposure resulted in modest increases in lavage fluid neutrophil numbers (14-fold increase), with concomitant increases in LDH (70%) and protein (80%). Somewhat surprisingly, the group ED_{150} for this dose was significantly less than the group ED_{150} for the saline-exposed rats, with 40% of the 1987 extract–exposed rats developing increased airway responsiveness compared to 17% of saline-exposed rats. However, it should also be noted two of six controls had unusually high values (i.e., they were quite ACh unresponsive) which shifted the 95% CI upward. It may also be relevant that the double dose 1987 extract–exposed rats not only received twice the metal exposure, but also received twice the mass of sodium and other extracted solutes. The alveolar injury (i.e., lavage fluid protein increases) and airway responsiveness changes may reflect the combined insult of extract-associated metals along with that of exposure to a hyperosmolar salt solution.

For an exploratory comparison of the suspect toxic components of the extracts, we elected to combine the data sets for all rats exposed for 24 hr to either the 1986 or the 1987 extract (and their corresponding saline controls). First, the group mean (± SE)

Table 2. Comparison of BAL fluid biochemical and cellular indices (mean ± SE) and airway responsiveness to ACh in saline- or 1986 extract–exposed rats at 24 hr postinstillation.

Parameters (units)	Saline	1986 extract 10%	1986 extract 40%
Group size	(*n* = 4)	(*n* = 4)	(*n* = 4)
Actual exposure (mg extract/rat)	0	0.25	1.0
Metal exposure (μg/rat)	0	0.34	1.37
BAL fluid biochemistries			
LDH (U/mL)	43.2 ± 7.4	68.7 ± 12	124 ± 12*
Total protein (μg/mL)	200 ± 41	236 ± 33	308 ± 20
BAL fluid cell counts × 10^3/mL			
Total cells	59.4 ± 9.7	151 ± 16	300 ± 52*
Macrophages	53.5 ± 9.0	40.8 ± 7.8	54.4 ± 12
Neutrophils	4.2 ± 1.5	105 ± 13.4	245 ± 42.3*
Eosinophils	0.0 ± 0.0	0 ± 0	0 ± 0
Lymphocytes	1.7 ± 0.8	4.5 ± 2.4	1.4 ± 0.9
ACh EC_{150} arithmetic mean	6.95 ± 3.1	5.7 ± 1.3	5.3 ± 1.4

Asterisk (*) indicates significantly different than saline-exposed control rats.

Table 3. Comparison of BAL fluid biochemical and cellular indices and airway responsiveness to ACh in saline- or 1987 extract–exposed rats at 24 hr postinstillation.

Parameters (units)	Saline	1987 extract, double dose
Group size	(*n* = 6)	(*n* = 5)
Actual exposure (milligrams extract per rat)	0	5.0
Metal exposure (micrograms per rat)	0	0.93
BAL fluid biochemistries		
LDH (U/mL)	33.3 ± 1.9	56.8 ± 6.2*
Total protein (μg/mL)	205 ± 32.1	369 ± 24*
BAL fluid cell counts × 10^3/mL		
Total cells	66.4 ± 14	109 ± 12.1*
Macrophages	53.8 ± 10	43.9 ± 7.8
Neutrophils	4.3 ± 2.0	61 ± 12*
Eosinophils	0.09 ± 0.09	0.22 ± 0.22
Lymphocytes	8.2 ± 2.9	3.8 ± 1.9
ACh EC_{150} arithmetic mean	8.6 ± 1.6	4.4 ± .04*
Upper 95% CI	12.8	
Lower 95% CI	4.5	
% < lower 95% CI	17% (1/6)	40% (2/5)

Asterisk (*) indicates significantly different than saline-exposed control rats.

response was plotted against the mass of instillate (Figure 4A). Although there appeared to be a mass-dependent increase in the number of lavage fluid cells present after exposure to either the 1986 or 1987 extract, clearly the slope of the dose–response curve was much steeper for the 1986 extract–instilled rats. Thus, mass alone does not correlate with the overall extract-induced lung inflammatory response. Second, in Figure 4B the group mean lavage fluid cell count was plotted against the total metal exposure (i.e., the metal content of each exposure based on the metals depicted in Figure 3). Metal exposure for the 10, 40, and 100% 1986 extract exposures was 340, 1,370, and 3,400 ng/rat, respectively. Metal exposures for the 100 and 200%, 1987 extract exposure were 460 and 930 ng/rat, respectively, intermediate to that of the 1986 extract-metal exposures. As shown in Figure 4B, although the slope of the dose–response curves for rats receiving either the 1986 or 1987 extracts were in fact closer, the slope of the 1986 extract–exposed rats was still greater than that of the rats exposed to the 1987 extract.

Filter Extraction Experiments

Although the quantities of most metals were clearly greater in the 1986 and 1988 extracts, we confirmed, compared to other TSP samples we have analyzed, the overall metal content was very low (ranging from 50 µg As, Mn, or Sr up to 1,200 µg Zn/g of extract). As with the initial ICP–AES data, this observation was problematic in light of the fact these extracts were derived from TSP filters collected on relatively dirty winter days, at least for the 1986 and 1988 filters. To confirm the average PM loading on the filters selected for this study was in fact representative of the average PM burden during the winter months in question, we first used the individual filter mass and air flow information to calculate the approximate mean daily ambient TSP concentrations for the days these filters were collected. In addition, because PM_{10} is estimated to account for 55% of the TSP material by mass (*10*), we calculated the corresponding mean PM_{10} concentration for the same days (Table 4). For comparison we then used data from the AIRS*Data* air pollution database of the U.S. EPA to calculate the mean (± SE) monthly PM_{10} concentrations for January, February, and March; and the corresponding 3-month winter PM_{10} concentrations at the Lindon monitoring site for 1986, 1987, and 1988 (Table 3). We concluded the calculated PM_{10} concentrations for the filters used in the present study were in close proximity to the actual 3-month average PM_{10} concentrations that occurred in the valley for each year.

So the question remained as to why metal concentrations were so low in the extracted material. Either the metal content of the PM deposited on the selected filters was indeed low or, alternatively, the water-based extraction and/or lyophilization steps used on these glass hi-vol filters failed to remove PM-associated metals, or the metals were lost during processing and resuspension. Because we were using the extracts, in part, as surrogates of the actual PM accumulated in the Utah Valley, it was critical to better understand how the extracted material was related to original TSP material deposited on the filters. By way of review, carbonaceous material in ambient PM is present in one of two forms, either as elemental carbon (i.e., carbon black or soot) or as a variety of organic carbon species, including polycyclic aromatic hydrocarbon compounds and perhaps more lower boiling point materials. Recognizing the hi-vol filters used in this study were designed primarily to trap suspended particles, it is unlikely highly volatile organic compounds were effectively captured on this type of filter. However, some organic (as well as inorganic) materials may adsorb to the elemental carbon matrix of soot. Thus, high-boiling-point organic and semivolatile compounds adsorbed to soot may have been present. The water solubility of such agents is generally quite low, although certain of the more soluble compounds (e.g., acid organics) may have been recovered using our water extraction procedure. Because these filters were stored for nearly 10 years prior to extraction, it is difficult to predict whether such compounds would remain soluble. In any event, elements such as transition metals should have been adequately retained on the filters over this period of time.

To better define which elements in the extract were likely from deposited PM and which were simply material removed from the filters, we obtained new (unexposed) glass filters similar to those used by the State of Utah. However, these filters were not from the same period of production nor were the filters stored for the same period of time. As before, we extracted the entire filter using a 96-hr water-based extraction procedure. Additionally, to assess for loss during lyophilization, we directly analyzed half the supernatant using ICP–MS and lyophilized half before resuspension in 1 M HCl and ICP–MS analysis.

Results indicated that for most metals, very minor quantities were present in the water-based extract from an unexposed glass filter. However, even prior to lyophilization, the pH of the liquid extracted from the unexposed filter was quite alkaline (pH 9–10). Furthermore, the extract contained a large amount of sodium. Sodium content was not affected

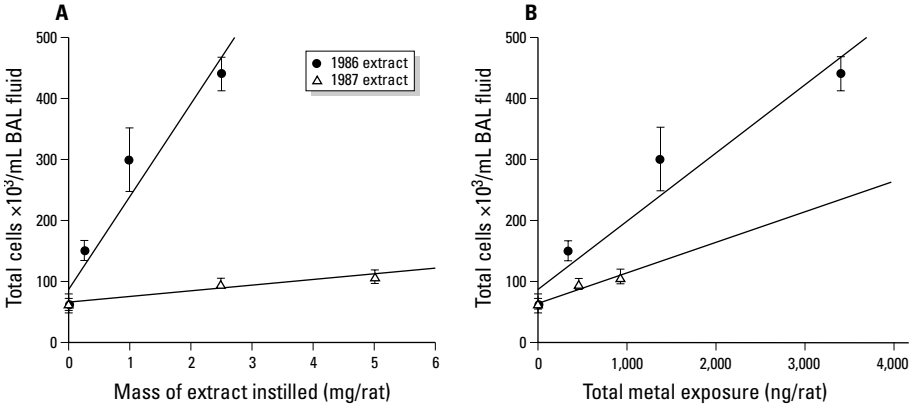

Figure 4. Comparison of the group (± SE) responses 24 hr after intratracheal instillation of either saline (n = 4, 6, or 12/group), the 1986 (plant on) extract (n = 4 or 12/group), or the 1987 (plant off) extract (n = 5 or 12/group) at different doses. (*A*) Total number of cells present in BAL fluid is plotted against the total mass of instillate (i.e., milligrams extract per rat). (*B*) Total number of cells present in the BAL fluid is plotted against the corresponding metal exposure (i.e., nanograms metal per rat).

Table 4. Comparison of monthly and 3-month averages (mean ± SE) for PM_{10} concentrations in the Utah Valley to TSP concentrations for the filters selected in the present study.

Parameter	1986	1987	1988
TSP filters used in the present study			
Total possible TSP collected on 12 filters (mg)	2,770	1,320	2,130
Total mass of extracted material (mg)	429	343	432
Mean TSP concentrations (± SE) (µg/m^3)	133 (± 27)	64 (± 14)	98 (± 17)
Estimates of PM_{10} based on the TSP content	73	35	54
AIRS*Data* for Lindon, Utah			
January PM_{10} concentrations (µg/m^3)	121 (± 20)	57 (± 5)	105 (±10)
February PM_{10} concentrations (µg/m^3)	30 (± 3)	39 (± 4)	100 (±11)
March PM_{10} concentrations (µg/m^3)	30 (± 4)	25 (± 2)	33 (± 2)
3-month average PM_{10} concentration	58 (± 8)	40 (± 3)	80 (±6)

by lyophilization. However by multiplying the amount obtained from 1 hi-vol glass filter × 12 filters, the calculated sodium value was similar to that present in the 1986–1988 extracts. Because we had previously determined the chloride content of the extracts using NAA analysis, we then calculated that 8–13% of the sodium extracted from the filters was present as NaCl. We further assumed the remaining sodium was present as Na_2CO_3 because sodium carbonate is commonly used in the manufacturing of glass products. Although typically Na_2CO_3 is present in hydrated forms, it is likely most of the water was removed during lyophilization. It is relevant that when Na_2CO_3 is placed in an aqueous solution, it is strongly alkaline.

On the basis of these estimates, we next calculated the amount of material in the extracts likely to be derived from the glass filter itself (i.e., the mass attributable to NaCl and Na_2CO_3) (Figure 5). In addition we assumed Ca, Mg, and K were present complexed with CO_3^- anions because Ca carbonate (i.e., limestone), K_2CO_3 (i.e., potash), and various forms of Mg carbonate are used during steel production. This is consistent with the fact that the NaCl and Na_2CO_3 extract content was fairly constant across the 3 years, whereas the Ca, Mg, and K content was higher in the 1986 and 1988 extracts obtained while the steel mill was operational (Figure 5).

With these estimates we were able to account for nearly 90% of the mass of the extracted material. Of primary importance, total sulfate, non–sodium cation content (Ca, Mg, and K) and total metal content of the 1986 and 1988 extracts were clearly higher than the 1987 extract. It was also apparent much of the extracted mass (60–70%) was attributable to sodium-based salts derived from the glass filter. The presence of large amounts of filter-derived salts makes it inappropriate to directly compare the concentrations of metals in our extracts to that of other TSP samples (i.e., baghouse-collected samples). For a more legitimate comparison we first calculated the total sulfate content (milligrams) and individual metal content (micrograms) for each extract. These values were then divided by the total amount of TSP material originally deposited on the filters (Table 5). As compared to the National Institute of Standards and Technology (NIST) TSP sample no. 1648, depending on the metal in question, the content in these extracts was still 6- to 300-fold lower than would be anticipated.

In another filter extraction experiment we obtained a hi-vol glass filter containing TSP material collected in 1983 at the Roy, Utah monitoring site north of Salt Lake City. We extracted triplicate filter sections using a stringent acid wash (1 M HCl) to determine the total amount of the sulfate and key metals present on a comparable TSP-loaded glass filter. For comparison, triplicate sections of the same filter were extracted using the previous water washing extraction. Again, the water-based supernatant was analyzed before and after lyophilization. Results indicated that essentially all of the sulfate present on the filter was effectively removed with a water extraction and that sulfate content was not affected by lyophilization. However, in comparison to a stringent acid washing of the same filter, the water-based extraction with a resultant pH of 8–9 failed to recover the majority of the PM-associated metals. By comparing the water-soluble to acid-soluble ratios of each metal, if one assumes the acid extraction removed all of the PM-associated metal, the water-based extraction only recovered approximately 3% of Cu and 1% or less of the Zn, Fe, Pb, Mn, and Ni relative to what was present in the acid-extracted sample. These results explain why the overall metal content of the 1986 and 1988 water-based extracts was quite low despite the fact that these filters were collected on relatively dirty winter days. Studies are in progress to determine whether more stringent acid washing would further increase removal of metallic elements from the matrix of a new (unexposed) filter as well.

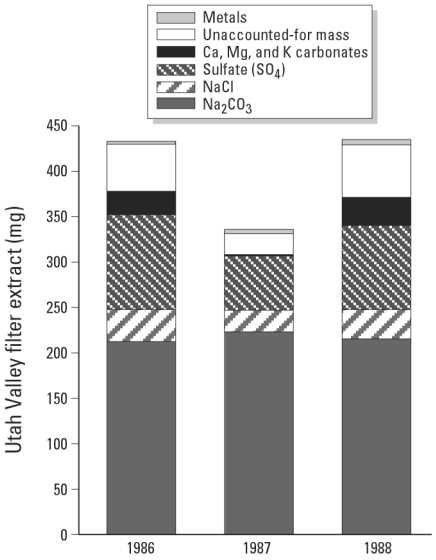

Figure 5. Comparison of the relative amounts of sodium-based salts; sulfate; Ca, Mg, and K carbonates; and total metal present in these extracts that were obtained by water extraction of 12 individual TSP-containing glass hi-vol filters per year. The filters were collected in the Utah Valley during the winter of 1986 (plant on), 1987 (plant off), or 1988 (plant on again).

Table 5. Comparisons of extract sulfate and metal content to NIST TSP sample no. 1648.

Total content per gm of TSP material collected	Utah extracts 1986	1987	1988	NIST no. 1648 (St. Louis)[a]
Milligrams sulfate per gram TSP	38.1	52.0	43.2	143
Micrograms metal per gram TSP for				
Copper	55.2	22.0	140.0	370
Zinc	74.0	6.8	13.1	3,560
Iron	26.4	3.03	54.0	8,910
Lead	24.2	3.0	38.0	ND
Strontium	14.1	3.8	29.1	ND
Arsenic	9.4	7.6	11.7	ND
Manganese	4.3	3.8	8.9	ND
Nickel	4.3	1.2	4.7	110
Vanadium	0.72	0.76	0.47	110

ND, not determined.
[a]Data derived from Costa and Dreher (*8*).

Discussion

Changes in the operating status of the Geneva Steel plant between 1986 and 1988 provided an unusual opportunity to investigate the potential link between PM excursions and effects on human health. In 1989, Pope published a seminal study describing the striking parallel between hospitalization rates for airway and respiratory ailments such as asthma and lung infections, particularly among young adolescents, with changes in Utah Valley ambient PM levels. Pope observed that PM concentrations were relatively high, low, and then high again before, during, and after the steel mill was shut down because of a labor strike (*3*). Since that first study, additional studies assessing the contribution of Utah Valley PM to adverse health effects have been aided by the generally low ambient levels of ozone, sulfur dioxide, and strongly acidic aerosols and the fact that the population studied from along the Wasatch front metropolitan areas of Utah Valley comprises 90% nonsmokers. As such, several additional studies have provided convincing and coherent evidence that industrially derived PM emissions trapped in stagnant winter inversions along the mountain front have a major impact on the cardiopulmonary health of Valley residents. Reported effects ranged from impaired lung function and school absenteeism to hospitalizations and mortality (*3,11–15*).

In this report we observed that administration of equal masses of water-based extracts from plant on 1986 and 1988 filters (but not plant off 1987 filters) to healthy adult rats resulted in acute but transient pulmonary injury and neutrophilic inflammation. Likewise, Ghio and Devlin observed healthy humans instilled via bronchoscopy with similarly extracted material also developed acute lung lobe injury and neutrophilic inflammation after exposure to 1986 or 1988 (but not 1987) extracts (*16*). Furthermore, direct exposure of human alveolar macrophages to the 1986 extract resulted in an immediate oxidative response, decreased phagocytic function, and increased apoptosis, and

overnight exposure to 1986 or 1988 extracts appeared to inhibit macrophage oxidant activity (*17*). Such effects could impair pulmonary host defense mechanisms. In other *in vitro* studies, investigators reported exposure of human airway epithelial cells to extracts from 1986 and 1988 (but not 1987) resulted in epithelial injury and release of proinflammatory mediators. These effects appeared to correlate well with acellular *in vitro* oxidant production of thiobarbituric acid reactive substances (TBARS) (*18*).

In this present study we further observed that CD rats instilled with the 1986 or 1988 extracts developed histologic evidence of alveolar and airway cell damage, and that up to 50% of rats developed transient but significant increases in nonspecific airway responsiveness, compared to only 17 and 25% of saline- or 1987 extract–exposed rats, respectively. Diagnoses of inflammatory lung diseases and exacerbations of asthma were the primary criteria of the plant-on/off period associated hospitalizations in the seminal study of Pope (*3*). By inference, had asthmatic individuals (i.e., individuals with pre-existing airway inflammation and a propensity to develop bronchoconstriction) been exposed to the 1986 or 1988 extracts, increased symptomatology related to exacerbation of airway inflammation and/or bronchoconstriction may have been more likely to occur.

As the primary contributor of industrial-related PM in the Valley, emissions from the Geneva Steel Mill have always been highly suspect as causative. There has remained doubt, however, due to the lack of direct biologic evidence supporting plausible causality of the emissions and its components. Acquisition of ambient TSP filters from the Lindon, Utah, airshed over the 1986–1988 winters allowed for the use of animal toxicity testing to better substantiate the link between wintertime PM excursions and effects on respiratory system health. The archived filters provided PM-like material, albeit in limited quantities, for use in *in vivo* animal toxicology studies and for characterization of PM changes in the affected airshed over time. Unfortunately, direct removal of particulate material from loaded high-vol filters is extremely difficult, if not impossible. The process is complicated by the inherent sticky nature of the collected particulate material, the tremendous surface area and interstices of the filter matrix, and the fact that components of the filters contribute to the extracted material. To date, various physical attempts at gentle scraping of the filter surface, or tapping or acoustically pulsing the filters to dislodge deposited particles have either failed to produce adequate PM recovery or have resulted in destruction of the filter itself. Thus, water extraction to obtain readily soluble (and potentially bioavailable) materials as we have used on bulk PM samples seemed a reasonable approach to yield samples appropriate for toxicologic assessment. However, due to the alkalinity related to Na_2CO_3 from the filters, we now postulate that even if metallic or acid organic substances associated with the PM were solubilized during water washing, they quickly precipitated out of the alkaline solution and were in essence readsorbed to the filter matrix. This possibility is supported by studies on soil bioremediation investigating metal sorption onto clay minerals. Results indicate cadmium, Cu, and Zn adsorption to the minerals kaolin and montmorillonite are highly pH dependent. Under alkaline conditions (pH 9–10), adsorption is greater than 80%, whereas at a pH < 6, adsorption falls to 20% or less depending on the metal in question (*19*). We are currently investigating the extent of PM-associated metal recovery from hi-vol filters as a function of pH of the extracting liquid medium. If we can improve upon metal recovery, future studies using extracts from archived or contemporary hi-vol filters may provide even more robust coherence between toxicologic studies and associated epidemiologic health findings.

So the question remains as to which elements or biophysical properties of the 1986 and 1988 extracts were in fact driving the pulmonary toxicity observed. Endotoxin content is unlikely because the extracted endotoxin levels were well below that considered to be proinflammatory in healthy CD rats (*7*), interactions between endotoxin and metal exposure notwithstanding (*20,21*). In light of the brownish discoloration of the 1986 and 1988 suspensions, the influence of as yet unidentified soluble organics (i.e., acid organics) cannot be discounted at this time. However, of the extract components identified to date, metal content is a probable causative agent driving the pulmonary toxicity of the 1986/1988 extracts, despite the fact that only ≤ 1% of the extract by mass was metals. We now recognize the low quantity of metal in these extracts was related to the fact only minor amounts of the original PM-associated metals were effectively extracted, and the metal concentrations in our extracts were further diluted by the recovery of filter-derived salts. We speculate that had more complete metal recovery occurred, the increase in PM-associated metals in the 1986/1988 extracts would have increased disproportionately to the 1987 extract, and thus the toxicity of the 1986/1988 extracts would have increased disproportionately as well. By extension, any risk assessment inferences based on this and related reports on the potential differences in the toxicity of ambient Utah Valley PM when the steel mill was or was not operational may be gross underestimates. Much remains to be proven however.

We are currently attempting to replicate the *in vivo* pulmonary toxicity of these extracts by intratracheally instilling various metal and/or salt mixtures in rats. Specifically, we are investigating acute effects of *a*) alkaline solutions (pH ≈ 10); *b*) NaCl + Na_2CO_3 (hyperosmolar) solutions; *c*) steel mill–associated cationic salts (i.e., Ca, Mg, and K carbonate); *d*) metal mixtures specific to that found in each year of the extracts; and *e*) combinations of these exposures. Because the pH and sodium-based salt content of the 1987 extract was comparable to that of the 1986 and 1988 extracts, it is unlikely that either of these factors alone will result in significant pathology. However, the high sodium carbonate content of the extracts may serve to explain why these extracts were alkaline, whereas many ambient TSP samples are neutral or mildly acidic in an aqueous suspension. In our attempts to replicate the pulmonary toxicity of these extracts, it may also be relevant that transition metals in these extracts are likely to be complexed with carbonate anions rather than occurring as relatively soluble metal sulfates. Pulmonary toxicity, deposition, and translocation kinetics of soluble metal sulfates in the lung can be quite different than that of more insoluble metal complexes such as oxides (*22*). To date, most studies exploring the role of metals in PM toxicity have used readily soluble metals (typically SO_4^{2-}) and have attributed toxicity to the soluble metals apart from anionic speciation (*23,24*). However, incidental evidence suggests alkalization of metals may result in enhanced toxicity of the metal mixture through undefined mechanisms (*23*).

By doubling the 1987 (plant off) extract instillation dose, we unexpectedly observed that rats developed significant lung injury, inflammation, and increased airway responsiveness. Knowing now that the 1987 extract also contained large quantities of salts, mostly in the form of sodium and presumably carbonates based on the use of glass fiber filters, we speculate these effects were substantially related to hyperosmolarity of the instilled extract suspension at the higher 5 mg/rat dose. Previous studies have demonstrated hyperosmolar insults may result in increased airway responsiveness in rabbits (*25*) and increased vascular permeability (and possibly neuropeptide release) in rats (*26*). In addition, intracellular fluid loss in lung epithelial cells (as could occur after instillation of the rats with a hyperosmolar salt solution) is thought to contribute to exercise-induced bronchoconstriction, late-phase asthmatic responses (*27*), and increased chemokine expression in airway epithelial cells (*28*).

Finally, we observed that although both the 1986 and 1988 extracts elicited lung injury and inflammation, the 1986 extract

elicited a relatively greater acute inflammatory response. Why was this so? Simple evaluation of the total metals present (Figure 3) indicates that if anything, the 1988 extract had approximately 10% more total metals. However, knowing individual metals often have unique pulmonary cellular effects, we speculate the overall toxicity of a given extract reflected not only the total metal content, but also the specific combination of metals present. As evident in Figure 4B, comparison of extract total metal content only partially explained the pulmonary inflammatory response induced. One possible explanation for the greater slope of the 1986 extract dose–response curve is that the metal combination in this plant on extract was more proinflammatory than the metal combination present in the plant off 1987 extract. In like fashion, previous metal mixture studies in CD rats indicated the pulmonary toxicity associated with instillation of injurious quantities of Ni was diminished by co-instillation of Fe and/or V (*23*). In airway epithelial cells, V-induced cytotoxicity appeared to be diminished by the presence of Fe (*29*) whereas Cu-induced cytotoxicity was enhanced by co-exposure to nontoxic quantities of Zn (*30*). In still other *in vitro* studies, the oxidative burst induced in alveolar macrophages exposed to residual oil fly ash samples of varying metal composition was greatest in cells exposed to fly ash containing the most leachable V. This response, however, was diminished in cells exposed to V plus Ni-containing fly ash (*24*). Thus, metal interactions, both antagonistic and synergistic, may play an important role in defining the pulmonary toxicity of specific PM samples.

Collectively, the present and related studies using humans and animals with *in vivo* and *in vitro* methodologies demonstrate that exposure to equivalent masses of the water-based extracts of local ambient PM filters from 1986 and 1988 (but not 1987) results in acute pulmonary injury and inflammation and possibly effects on pulmonary immune and constrictor responses. Thus, the experimental toxicity of these extracts is in good accord with the cross-sectional epidemiology studies reporting adverse health effects in Utah Valley residents. The ability to replicate in the laboratory what was determined from epidemiology studies has provided *a*) coherent and independent confirmation of the health effects findings, and *b*) a proof of concept for the use of empirical experimental studies to assess and perhaps predict likely ambient PM adverse health effects. The conceptual linkage between studies of human populations and empirical laboratory studies validates the further use of controlled human, animal, and *in vitro* studies designed to define underlying mechanisms to refine risk assessment paradigms. The strengths imparted by an integrated approach of epidemiologic, human, animal, and *in vitro* studies can only augment quantitative risk evaluations and regulatory decision making.

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